USE OF STAM CELL IN THE TREATMENT OF DIFFICULT WOUNDS

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SUMMARY

Purpose: *Tissue engineering has been a topic of extensive research over the last years. Successful permanent wound coverage remains one of the major problems in patients with large wounds and most of surgeons have focused on the development and execution of new techniques to treat difficult wounds. A lot of reports have been published on the clinical use of cultured autologous keratinocytes (CAK) but the unsatisfying short and long term results, concerning mechanical stability and scarring, demand for alternatives.* **Methods:** *Eleven consecutive patients were treated using cultured autologous fibroblasts (CAF) followed by skin graft in resurfacing woundst.* **Results:** *We observed excellent results in all the patients. Hypertrophic scar was noted in two patients. Patient satisfaction was also very positive.* **Conclusions:** *The importance of CAF and their transplantation onto a wound bed to improve the rate of wound healing is emphasised. The fibroblasts on the wound bed can secrete various cytokines, especially growth factors, which control cell proliferation, induce angiogenesis and modify the inflammatory process. We observed a successful cosmetic outcome using this technique providing a good restoration of tissue continuity and function.*
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KEY WORDS

Wound, cultured fibroblasts, cultured keratinocytes, skin autografts, tissue engineering

INTRODUCTION

Plastic surgery is the surgery of wounds. Most of surgeons have focused on the development and execution of new techniques to treat difficult wounds. While our subspecialty has made significant advances, other groups have contributed substantially to non-surgical methods of treating wounds.

The rate of wound healing is limited by the vascular supply available, the formation of new capillaries and matrix molecules. These events are heavily influenced by locally acting growth factors. Successful permanent wound coverage remains one of the major problems in patients with large burns (>60%) (1). The only available sites may be located on areas of the body which are unsuitable for harvesting, such as the face, the hands or the perineum. Because these limitations to wound coverage, investigators have continued to develop methods of permanent wound closure.

Since the mid 1970's when Rheinwald and Green (2) developed a method for study of human keratinocytes function by sustaining their growth in vitro, scientists and clinicians have intensified their investigation of the development of cultured autologous keratinocytes (CAK) as a functional burn wound covering. It has been over 25 years since the first patients were grafted with cultured keratinocyte grafts and the subject has attracted much enthusiasm in both clinical and experimental literature (3).

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A variety of wounds have been successfully resurfaced with cultured autogenic keratinocytes, but comparative assessment of the safety and effectiveness of skin substitutes are still lacking. Problems includes sloughing, blistering, scarring, wounds contraction (1), mechanical fragility (4-6) and a highly susceptibility to infection (7-11). Biologic factors that require consideration include, but are not limited to: rates of engraftment, functional outcome and cosmesis. Consequently, socioeconomic impacts of skin substitutes on recovery are not understood sufficiently well to provide justification for their routine use (12).

There have been a lot of reports published on the clinical use of CAK, this study describes the results and the efficacy with the use of cultured autologous fibroblasts (CAF) followed by skin graft in resurfacing wounds. The complex extracellular matrix, secreted by the fibroblasts, improves adhesion properties of keratinocytes to the wound, important in maintaining the integrity and function of the skin.

This new engraftment technique to resurface the wounds has been successfully applied on giant nevus excision, chronic ulcerations, trauma and tumour resection.

MATERIALS AND METHODS

Patients

This technique has been used to treat 11 consecutive patients (mean age 32.6 years), with a variety of wounds and aetiologies, between January 2004 and September 2005. The gap size ranged from 9.8 to 230 cm^2 (mean 79.6 cm^2). Three patients had trauma resection (2 women an 1 man) (Fig. 1), 1 patient had tumor resection (woman), 1 patient had burn's scar (Fig. 2), 3 had chronic diabetic ulcerations (2 men and 1 woman) and 3 patients had giant nevus excision (1 boy and 2 girls).

All patients were required to give prior written consent. Most full-thickness wounds were excised to fascia, covered with CAF and successively with traditional skin graft.

Technique

Blood samples from all donors were tested for any infection disease, including hepatitis and human immunodeficiency virus. During the first operative procedure an area of the body was selected as the donor site, approximately 4x3 cm^2 in size (Fig. 3A). Samples were harvested under sterile conditions and taken immediately to the laboratory specializing in the creation of bioengineered tissues (Laboratori TISSUEtech™, Abano Terme, Italy) (Fig. 3B), where epidermal keratinocytes and fibroblasts were isolated by standard methods. On the twenty-first day the autologous fibroblasts grown on Hyalograft 3D (Fidia Advanced Biopolymers, srl - Abano Terme, Italy) were grafted directly onto the wound bed. This dermal-like graft was allowed to take and to stimulate the formation of a well-vascularized neodermis capable of receiving the skin graft (13-21). Hyalograft 3D™ is a dermal substitute consisting of autologous fibroblasts on a three-dimensional matrix of HYAFF®, a biomaterial derived from hyaluronic acid. Fibroblasts adhere to the fibres and secrete proteins, glycoproteins and matrix molecules to form an extracellular matrix (20-25). The grafted areas were covered with degreased petrolatum gauze and wrapped in sterile bandages. The external medication was changed and the grafts monitored after 4 days. In these patients, in the 7-10 days post-graft, it was not possible to use any type of disinfectant solution because of the great delicacy of the keratinocytes. In 6 patients the CAF were applied once to obtain granulation tissue, in four patients they were placed a second time two weeks later. After 10 days a widely expanded meshed grafts (3:1 mesh ratio) were placed over full-thickness wounds. The receiving areas were disinfected with chlorhexidine solutions and then washed in copious amounts of sterile physiological solution. The graft areas were covered in degreased petrolatum gauze and wrapped in sterile bandages. In all patients complete closure was achieved with a single application of skin graft.

Complete closure was defined as the completely epithelized state at which the patient was able to have a shower. The time required for complete

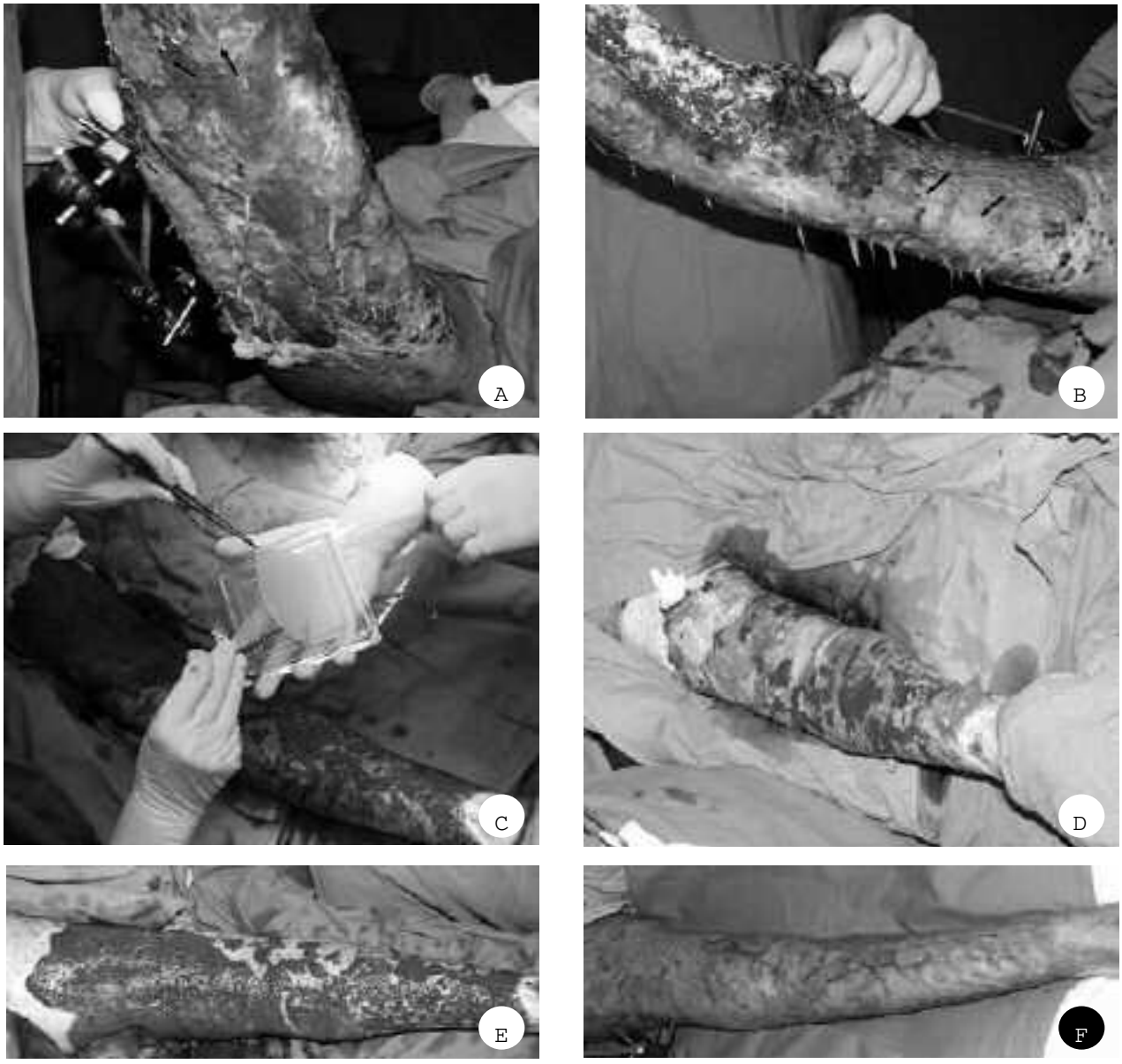

Figure 1. *A, B) patient came to us already treated with meshed skin graft showing evidence of unsuccessful taking (arrows); C, D) we covered with CAF; E) after 10 days, new granulation tissue covered the surface of the wound; F) after 33 days, complete reepithelialization was achieved.*

healing ranged from 33 to 41 days after treatment (mean 37.6 days).

Results

Qualitative outcome was evaluated beginning on day 14 and proceeded as long as patients returned to the hospital for subsequent examination. We observed excellent results. Parameters of qualitative outcome included the following: erythema, pigmentation, epidermal blistering, surface texture and raised scar. The results were valued using a visual scale that measures the quality of the outcomes with a score of 0 being the worst and 10 being the best (mean scores of 8.9). Hypertrophic scar was

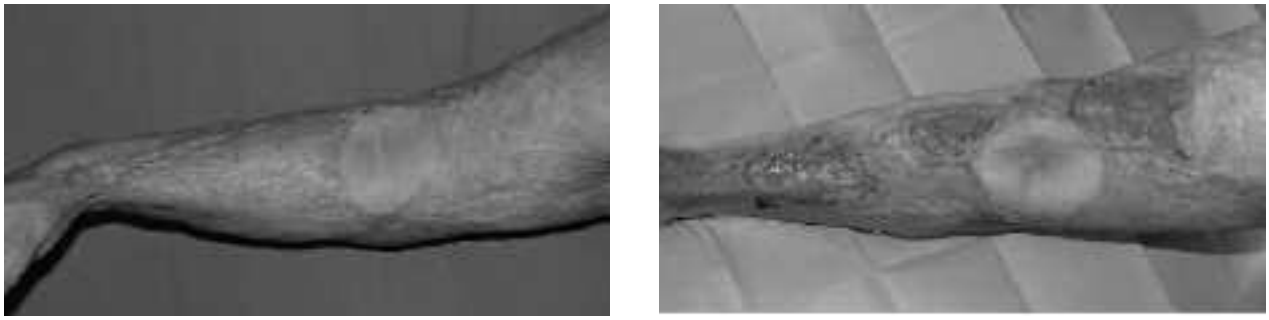

Figure 2. *Wound excised to fascia and covered with CAF and successively with meshed skin graft.*

noted in two patients. However, neither the patients nor the surgeons found them to be clinically significant. There were no cases of recurrence or infection during the follow-up period. Patient satisfaction was also very positive.

DISCUSSION

There have been numerous reviews that discuss the field of tissue engineering in general. The ultimate objective for skin substitutes is restoration of the anatomy and physiology of ininjured skin. The full potential for engineering of skin substitutes has not yet been realized. This review is not intended to be exhaustive, but rather to provide an overview of tissue engineering in the context of clinical plastic surgery, illustrated by developments in several wound treatments. We report our experience with these grafts, which are designed to furnish a dermal replacement for the full-thickness wounds.

Epidermal cells consist predominantly of keratinocytes (95-97%), dermal cells include fibroblasts and most of the dermis consist of extracellular matrix (collagens, elastin, reticulin, poly-saccharides) that provides the majority of the mechanical strength to the skin (26). Morbidity is characterized by loss of tissue structure and function, full recovery of skin function may not be expected without full restoration of all cell types in ininjured skin substitutes.

Both clinical and laboratory studies show that the growth of meshed skin autografts depends most critically on the nature of the wound bed (27). One year after grafting the epidermis may be fragile, being easily abraded by mild trauma and prone to spontaneous blistering.

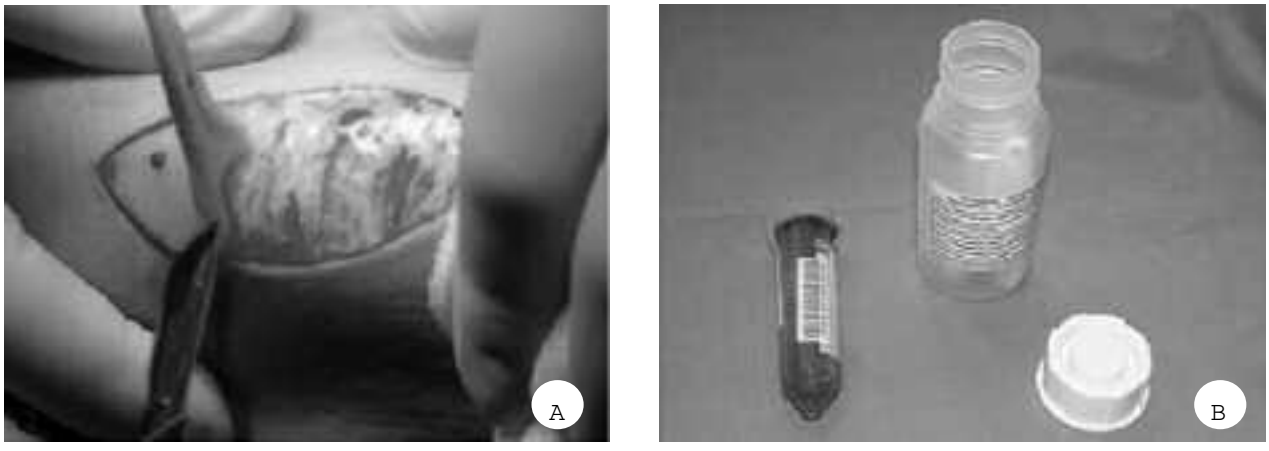

Figure 3. *Biopsy harvested under sterile conditions and taken to the laboratory.*

The use of mesh graft has improved the ability of the surgeon to achieve permanent coverage of large full-thickness wound areas, but the results are often suboptimal. There are several reports showing the lack of long-term durability and graft fragility. Epithelialization of mesh grafts interstices from grafts bridges eventually closes the wound but frequently results in skin that is thin and prone to breakdown in the early healing period, with decreased pliability and poor cosmetic appearance. Hypertrophic scar formation and wound contraction frequently occur in wounds, particularly when mesh ratios greater than 1:5 are used.

This technique brings the fibroblasts on the wound bed where they can secrete various cytokines, especially growth factors, which control cell proliferation, induce angiogenesis and modify the inflammatory process. Growth factor secretion is a function of live cells, so the ability of live fibroblasts in implants to colonize wound beds is a great importance (28, 29). Fibroblasts also produce extracellular matrixes, consisting of collagen, proteoglycans and other proteins, which fills the wound's hollows, thus improving the cosmetic outcome. Recovery of function and cosmesis allows return of patients to productive roles in society.

The small size of the biopsy needed to get the autologous grafts is particularly appreciated by the patients, who have to bear the scarring results.

We observed a successful outcome using this technique: adherence to wounds, high rate of engraftment, control of fluid loss and infection, absence of antigenicity and toxicity, reduced wounds contraction, mechanical stability and compliance.

However, this therapy needs a lot of time, because only when a sufficient amount of cells were grown the patient is admitted for the excision of the lesion or scars, that is why we decided to use it on patients with non extensive wounds. Additionally, tissues engineering is prohibited expensive. Nonetheless in the future centralizing the manufacture of cultured fibroblasts, in either a public or private health system, could bring down expenses.

Tissue engineering will potentially change the practice of plastic surgery more than, perhaps, any other clinical specialty, offering the natural "next step" in the evolution of plastic surgery (30, 31). Additionally, this is a branch of tissue engineering that doesn't use embryonic stem cells that are the targets of strong criticism as their use raises serious ethical issues (19).

Although the number of cases in this study is not sufficient yet and further investigation is needed to determine the number of fibroblasts surviving in the bed wound, we believe that this method provides a good environment for the treatment of difficult wounds.

References

1. Herzog SR, Meyer A, Woodley D, Peterson HD. Wound coverage with cultured autologous keratinocytes: use after burn wound excision, including biopsy follow-up. J Trauma 1998; 28: 195-8.
2. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 1975; 6: 331-43.
3. O'Connor NE, Mulliken JB, Banks-Schlegel S. Grafting of burns with cultured epithelium prepared from autologous epidermal cells. Lancet 1981; 75-8.
4. Odessey R. Addendum: multicenter experience with cultured epithelial autografts for treatment of burns. J Burn Care Rehabil 1992; 13: 174-80.
5. Desai MH, Mlakar JM, Mc Cauley RL, et al. Lack of long term durability of cultured keratinocyte burn wound coverage: a case report. J Burn Care Rehabil 1991; 12: 540-5.
6. Williamson J, Snelling C, Clugston P, Macdonald I, Germann E. Cultured epithelial autograft: five years of clinical experience with twenty-eight patients. J Trauma 1995; 39: 309-19.
7. Hansbrough JF, Boyc ST, Cooper ML, Foreman TJ. Burn wound closure with cultured autologous keratinocytes and fibroblasts attached to a collagen-glycosaminoglycan substrate. JAMA 1989; 262: 2125-30.
8. Pittelkow MR, Scott RE. New techniques for the in vitro culture of human skin keratinocytes and prospectives on their use for grafting of patients with extensive burns. Mayo Clin Proc 1986; 61: 771-7.
9. Boyce ST, Greenhalgh DG, Kagan RJ, et al. Skin anatomy and antigen expression after burn wound closure with composite grafts of cultured skin cells and biopolymers. Plas Reconstr Surg 1993; 91: 632-41.
10. Pellegrini G, Bondanza S, Guerra L, De Luca M. Cultivation of human keratinocyte stem cells: current and future clinical applications. Med Biol Eng Comput 1998; 36: 778-90.

11. Boyce ST, Hansbrough JF. Biologic attachment, growth, and differentiation of cultured human epidermal keratinocytes on a graftable collagen and chondroitin-6-sulfate substrate. Surgery 1988; 103: 421-31.
12. Rue LW, Cioffi WG, McManus WF, Pruitt BA. Wound closure and outcome in extensively burned patients treated with cultured autologous keratinocytes. J Trauma 1993; 34: 662-7.
13. Scalise A, Pierangeli M, Di Benedetto G, Sperti V, Bertani A. Cultured autologous fibroblast and keratinocyte grafts: applications in plastic surgery. Annals of Burns and Fire Disasters 2001; 14 (2).
14. Travia G, Palmisano PA, Cervelli V, Esposito G, Cascinai CU. The use of fibroblast and keratinocyte cultures in burns treatment. Annals of Burns and Fire Disasters 2003; 16 (1).
15. Boyce ST, Goretsky MJ, Greenhalgh DG, Kagan RJ, Rieman MT, Warden GD. Comparative assessment of cultured skin substitutes and native skin autograft for treatment of full-thickness burns. Ann Surg 1995; 222: 743-52.
16. Bannasch H, Momeni A, Knam F, Stark GB, Fohn M. Tissue engineering of skin substitutes. Panminerva Med 2005; 47: 53-60.
17. McGuire MK, Nunn ME. Evaluation of the safety and efficacy of periodontal application of a living dermal substitute. Comparison to the gingival autograft: a randomized controlled pilot study. J Periodontol 2005; 76: 867-80.
18. Wisser D, Steffes J. Skin replacement with a collagen based dermal substitute, autologous keratinocytes and fibroblasts in burn trauma. Burns 2003; 29: 375-80.
19. Ahsant T, Nerem RM. Bioengineered tissues: the science, the technology and the industry. Orthod Craniofac Res 2005; 8: 134-40.
20. Chan ES, Lam PK, Liew CT, Lau HC, Yen RS, King WW. A new technique to resurface wounds with composite biocompatible epidermal graft and artificial skin. J Trauma 2001; 50: 358-62.
21. Fimiani M, Pianigiani E, Di Simplicio FC, et al. Other uses of homologous skin grafts and skin bank bioproducts. Clin Dermatol 2005; 23: 396-402.
22. Compton CC, Gill JM, Bradford DA, Regauer S, Gallico GG, O'Conner NE. Skin regenerated from cultured epithelial autografts on full-thickness burn wound from 6 days to 5 years after grafting. Lab Invest 1989; 60: 600-12.
23. Compton CC, Regauer S, Seiler GR, Landry DB. Human Merkel cell regeneration in skin derived from cultured keratinocyte grafts. Lab Invest 1990; 63: 233-41.
24. deSerres S, Herzog SR, Meyer AA, Peterson HD. Techniques to accelerate the availability of human keratinocyte grafts. J Burn Care Rehabil 1989; 10: 469-75.
25. Woodley DT, Peterson HD, Herzog SR, et al. Burn wounds resurfaced by cultured epidermal autografts show abnormal reconstitution of anchoring fibrils. JAMA 1988; 259: 2566-71.
26. Boyce ST. Design principles for composition and performance of cultured skin substitutes. Burns 2001; 27: 523-33.
27. Hansbrough JF, Cooper ML, Cohen R, et al. Evaluation of a biodegradable matrix containing cultured human fibroblasts as a dermal replacement beneath meshed skin grafts on athymic mice. Surgery 1992; 111: 438-46.
28. Seung-Kyu H, Kyu-Jin C, Woo-Kyung K. Clinical application of fresh fibroblast allografts for the treatment of diabetic foot ulcers: a pilot study. Plas Reconstr Surg 2004; 114: 1783-9.
29. Middelkoop E, van den Bogaerdt A, Ulrich M. The role of fibroblasts in skin substitutes. Wound repair regen 2005; 13: A22.
30. Giannoudis PV, Pountos I. Tissue regeneration. The past, the present and the future. Injury 2005; 36 Suppl 4: S2-5.
31. Supp DM, Boyce ST. Engineered skin substitutes: practices and potentials. Clin Dermatol 2005; 23: 403-12.